

R E M A R K S

The Examiner is thanked for the courtesies she extended to the undersigned during telephone interviews on November 22, 2004, November 23, 2004 and December 2, 2004.

Claims 1 and 4 were amended by including features supported on page 6, line 12 to page 8, line 6 of the specification.

Claims 2, 5 and 17 to 19 were amended by including features supported on page 8, lines 7 to 14 and page 14, line 4 to page 15, line 5 of the specification.

Claim 10 was editorially revised.

New claim 20 is supported in the specification on page 8, lines 12 to 14.

It is noted that Item No. 5 at the bottom of page 5 of the Office Action indicated that claims 3, 6, 8 to 10 and 12 to 19 included allowable subject matter.

Claims 1 to 19 were rejected under 35 USC 112, second paragraph, for the reasons set forth in Item No. 2 on page 2 of the Office Action.

It is respectfully submitted that the above amendments to claims 1 and 4 serve to avoid the 35 USC 112, second paragraph rejection.

Withdrawal of the 35 USC 112, second paragraph rejection is therefore respectfully requested.

As recited in claim 1, the presently claimed invention concerns a method for quantitatively determining hydrogen sulfide or sulfide ions which comprises (a) adding to a sample containing hydrogen sulfide or sulfide ions, (i) metal ions or a compound which liberates the metal ions and (ii) a metal indicator which reacts with the metal ions and resultantly undergoes a color development, the color development being accelerated or inhibited by the hydrogen sulfide or sulfide ions, wherein complexes are formed by the reaction of the metal ions and the metal indicator, and (c) measuring the degree of color development of the metal indicator by determining the amount of the formed complexes and subtracting from said amount the amount of formed complexes when the metal ions are reacted with the metal indicator when no hydrogen sulfide or sulfide ions are present, whereby a decreased

amount of the formed complexes indicates an inhibition of color development and corresponds to the amount of the hydrogen sulfide or sulfide ions present in the sample, or an increased amount of formed complexes indicates an acceleration of color development and corresponds to the amount of the hydrogen sulfide or sulfide ions present in the sample.

As recited in claim 4, the presently claimed invention is also directed to a method for quantitatively determining a specific substance which comprises (a) adding to a sample containing a specific substance, (i) a component which acts on the specific substance so that the specific substance forms hydrogen sulfide or sulfide ions, (ii) metal ions or a compound which liberates said metal ions, and (iii) a metal indicator which reacts with the metal ions and resultantly undergoes a color development, the color development being accelerated or inhibited by the hydrogen sulfide or sulfide ions, wherein complexes are formed by the reaction of the metal ions and the metal indicator, and (c) measuring the degree of color development of the metal indicator by determining the amount of

the formed complexes and subtracting from said amount the amount of formed complexes when the metal ions are reacted with the metal indicator when no hydrogen sulfide or sulfide ions are present, whereby a decreased amount of the formed complexes indicates an inhibition of color development and corresponds to the amount of the hydrogen sulfide or sulfide ions present in the sample, or an increased amount of the formed complexes indicates an acceleration of color development and corresponds to the amount of the hydrogen sulfide or sulfide ions present in the sample.

Claims 1, 2, 4 and 5 were rejected under 35 USC 102 as being anticipated by Dabovic USP 6,107,100 for the reasons set forth in Item No. 2 on pages 2 and 3 of the Office Action.

The differences between the present invention and Dabovic are summarized in the following table:

	Present Invention	"Dabovic"
Object to be analyzed	thiol : no (In case homocysteine and cysteine, an enzyme produces hydrogen sulfide or sulfide ion.)	thiol : yes
	hydrogen sulfide or sulfide ion : yes	hydrogen sulfide or sulfide ion : no
Metal	Metal ion or Metal-ion producing reagent (water soluble)	Basic Zn-slurry (water insoluble)
Color development	Metal indicator reacts with metal ion to form a colored metal-chelating complex.	Na-nitroprusside, which is not a metal indicator, reacts with thiols. Metal indicator is defined as the reagent having an ability to form metal-chelating complex with metal ion, while Na-nitroprusside ($\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]2\text{H}_2\text{O}$) does not.
Resultant of Color development	Water soluble	Water insoluble
Measurement	The colored reaction mixture could be ready for direct colorimetric measurement.	The filtration- or solubilization (by EDTA)-step is required for colorimetric measurement.

The object analyzed in the presently claimed invention is hydrogen sulfide and/or sulfide ion, but not a thiol-chemical. Since thiol-chemicals do not exhibit any effects on the assay system, a quantitative and specific determination of hydrogen sulfide and/or sulfide ion can be performed, even in the presence of thiol-chemicals. In fact, the assay system in the presently claimed invention can include a thiol-chemical (2-mercaptoethanol with a concentration of 4mM in the 1st reagent, and as a final 2.4 mM; see Examples 2-4 on pages 20 et seq. of the present specification) as a supplemental reagent. In contrast to the presently claimed invention, thiol-chemicals are the object analyzed in Dabovic.

The following position is taken on page 4, lines 9 to 10 of the September 21, 2004 Office Action:

"..., it is in the examiner's position that the quantitative determination of hydrogen sulfide and/or sulfide ions is inherent in Dabovic's invention."

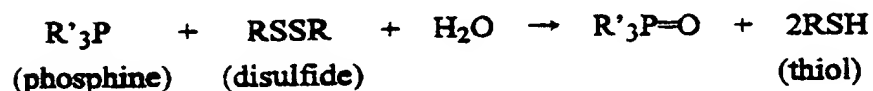
It is clear that the present invention is not inherent in Dabovic's invention for the following reasons.

In Photo 7 of Dabovic, the thiol-chemicals (β -mercaptoethanol, L-cysteine, dithiothreitol and DL-homocysteine) (listed in 2-5) develop colors clearly, while Na_2S (sodium sulfide) (listed in 8) does not. This is in contrast with the present invention in which the quantitative determination of Na_2S with a range of concentrations 0-100 μM in a sample is performed well by both an inhibition method and an acceleration method in Examples 1 and 2 of the present specification, respectively.

The following position is taken on page 4, lines 10 to 11 of the September 21, 2004 Office Action:

"As a result of the reduction of the disulfides to the thiol state, hydrogen sulfide ions are released."

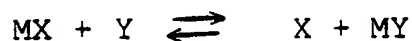
It is well-known to those of ordinary skill in the art that TCEP (tris(2-carboxyethyl) phosphine) utilized in Dabovic is a reducing reagent which transforms disulfides into thiols, and which does not produce either hydrogen sulfide or sulfide ion, which is shown by the following reaction:



In the presently claimed invention, the color development depends on the reaction in which a metal indicator reacts with metal ions to form a metal-chelating complex. In Dabovic, on the other hand, Na-nitroprusside, which does not react with metal ions, but rather reacts with thiol-residues, is the object to be measured. Na-nitroprusside is used as a color metric indicator for the quantitative development of sulfur, but it is not a metal indicator.

The chemical formula of Na-nitroprusside is $\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]2\text{H}_2\text{O}$, in which a metal element, namely Fe, is contained. Despite the existence of the metal element Fe, Na-nitroprusside itself does not develop color, and also has not been shown to exhibit any specificity to react with metal ions. Therefore, Na-nitroprusside is not categorized as a metal indicator. See the following English-language translation of a definition of a metal indicator set forth in the Japanese dictionary of physics and chemistry (IWANAMI RIKAGAKU JITEN, 5th Edition, p. 353, reference no. 456, April 24, 1998, Iwanami Syoten, Publishers) (a copy of the Japanese text is enclosed), which the applicants have provided:

"An indicator, which is utilized for determining the end-point in chelatometry of a metal ion. The metal indicator itself is capable of forming a metal-indicator complex with a color change. Moreover, the thus formed metal indicator complex must be less stable than the metal-chelate complex formed using a chelating agent when titrating with chelating agent Y after adding a small amount of metal indicator X to the solution including metal ion M, the following reaction is observed:



Initially the metal-indicator complex (MX) is present, exhibiting one color. Adding a chelating agent Y into the solution will detach the indicator X from metal the ion M. At an equivalence point, when all metal ions have been complexed with chelating agent Y, the color of the detached indicator (X) is observed. For example, when titrating with EDTA, Eriochrome Black T for a magnesium ion or 1-(2-pyridilazo)-2-naphtol(PAN) for a copper ion is suitable as a metal indicator. In general, the transition interval of a metal indicator is in between a narrow range of its pM value ($-\log_{10}[M^{n+}]$, whereas $[M^{n+}]$ means metal ion concentration) and that intervals is varied with the pH of the solution".

The fact that it is well known by those of ordinary skill in the art that Na-nitroprusside is not utilized as a metal indicator and is not a metal indicator is evidenced by searching the SIGMA-ALDRICH website, wherein information in respect of Na-nitroprusside, as well as Eriochrome Black T is found. As shown in the enclosed copy of such information, it is seen, while

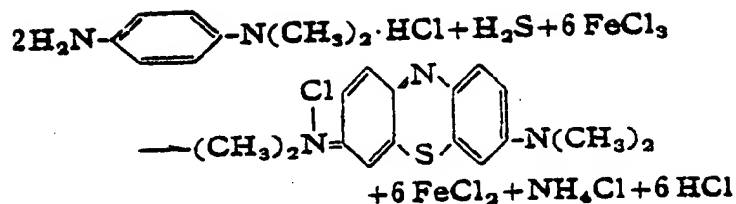
Eriochrome Black T is indicated as a substance suitable for a metal indicator, Na-nitroprusside does not have any indication.

As discussed above, the chemical formula of Na-nitroprusside is $\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]2\text{H}_2\text{O}$, in which a metal element Fe is contained. Since a metal indicator functions as an indicator of an extrinsically supplied metal ion, and the resultant solution develops colors depending upon the existence of the extrinsically supplied metal ion, Eriochrome Black T is supposed to exhibit such function without metal element in the molecule. On the other hand, despite the existence of a metal ion in the molecule, it is well known that Na-nitroprusside is not utilized for reacting with an extrinsically supplied metal ion to develop colors. Therefore, Na-nitroprusside should not be categorized as a metal indicator.

Claims 7 and 11 were rejected under 35 USC 102 as being anticipated by Tan et al. USP 5,985,540 for the reasons set forth in Item No. 3 on page 3 of the Office Action.

In Tan et al., N,N-dimethyl-p-phenylenediamine ("NDPD") (see column 26, lines 34 to 37) is oxidized by FeCl_3 to become methylene blue, which incorporates the element S derived from

hydrogen sulfide. The well-known reaction is illustrated as follows:



NDPD does not react with the metal Fe, so as not to form a colored metal chelating complex. Thus, NDPD is not categorized as a metal indicator (see the above definition of a metal indicator).

The following position is taken on page 5, lines 13 to 15 of the September 21, 2004 Office Action:

"..., it is the examiner's position that NDPD can be used as a color metal complex, absent any evidence to the contrary."

As discussed above, NDPD is oxidized by FeCl_3 to become methylene blue, which incorporates the element S derived

from hydrogen sulfide. In the reaction, NDPD and the resultant methylene blue do not have metal in the molecules.

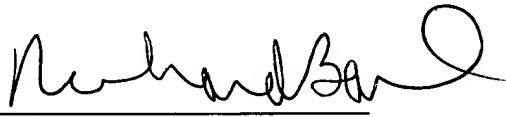
It is therefore respectfully submitted that applicants' claimed invention patentably distinguishes over the references. Withdrawal of the prior art rejections is respectfully requested.

Reconsideration is requested. Allowance is solicited.

If the Examiner has any comments, questions, objections or recommendations, the Examiner is invited to telephone the undersigned at the telephone number given below for prompt action.

Respectfully submitted,

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- Encs.: (1) copy of page 353 including reference no. 456 of the Japanese dictionary of physics and chemistry, IWANAMI RIKAGAKU JITEN (in Japanese), 5th edition, printed on April 24, 1998 by Iwanami Syoten, Publishers
- (2) copies of three pages from the SIGMA-ALDRICH website concerning Eriochrome Black T and sodium nitroprusside dihydrate

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E2377

Eriochrome Black T
suitable for metal indicator[Pricing and Availability](#)

Identifiers

Synonyms Mordant Black 11
Molecular $C_{20}H_{12}N_3NaO_7S$
Formula
Molecular Weight 461.38
CAS Number 1787-61-7
Colour Index 14645
Number
Bellstein Registry 4121162
Number
EG/EC Number 2172503
MDL number MFCD00003935

Properties

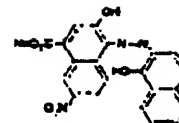
composition
Dye content ~65%

References

Merck Merck13, 3702
Bellstein Bell.16.IV.429
reference FT-IR22(3), 4095:A
RegBook1(2), 2751:F
Sigma FT-IR1(2), 540:C
Structure Index1:C:8

Safety Information

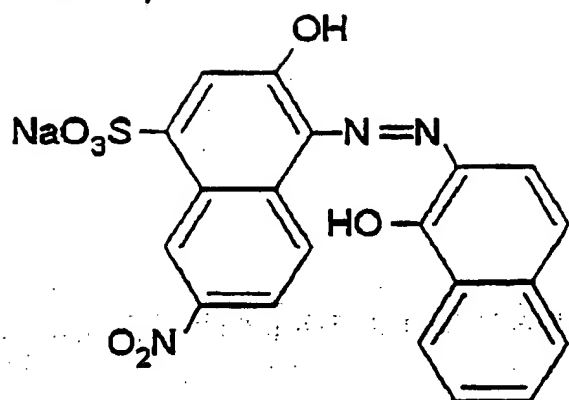
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RTCS QK2197000

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S0501

Sodium nitroprusside dihydrate

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Identifiers

Synonyms

SNP

Sodium nitroferrocyanide

Sodium pentacyanonitrosylferrate

Nitroprusside sodium

 $C_5FeN_5Na_2O \cdot 2H_2O$ $Na_2Fe(CN)_5NO \cdot 2H_2O$ Molecular
Formula

Molecular Weight 297.95

CAS Number 13755-38-9

EG/EC Number 2383739

MDL number MFCD00003506

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Description

Biochem/physiol
ActionsReleases NO *in vivo*, thus activating guanylyl cyclase, ADP-ribosyltransferase, and cyclooxygenase and inhibiting lipoxigenase. Induces vasodilation and inhibits platelet aggregation.

References

Literature

Azula, F.J., et al., Thrombin-stimulated phospholipase C activity is inhibited without visible delay by a rapid increase in the cyclic GMP levels induced by sodium nitroprusside. *Mol. Pharmacol.* 50, 367-379 (1996)Brune, B., and Lapetina, E.G., Activation of a cytosolic ADP-ribosyltransferase by nitric oxide-generating agents. *J. Biol. Chem.* 264, 8455-8458 (1989)Salvemini, D., et al., Nitric oxide-mediated cyclooxygenase activation. A key event in the antiplatelet effects of nitrovasodilators. *J. Clin. Invest.* 97, 2562-2568 (1996)Maccarrone, M., et al., Nitric oxide donor compounds inhibit lipoxigenase activity. *Biochem. Biophys. Res. Comm.* 219, 128-133 (1996)Noack, E., and Feilisch, M., Molecular mechanisms of nitrovasodilator bioactivation. *Basic Res. Cardiol.* 86, 37-50 (1991)Feilisch, M., *J. Cardiovasc. Pharmacol.* 17, S25 (1991)Rees, C.M., et al., Sites of vasodilation by inhaled nitric oxide vs. sodium nitroprusside in endothelin-constricted isolated rat lungs. *J. Appl. Physiol.* 77, 51-57 (1994)

Merck

Bellstein

Merck 13, 8722

Bell 2, IV, 74

Safety Information

Hazard Codes

T+, N

Risk Statements

26/27/28-32-50/53

Safety Statements

7-28-29-45-60-61

RIDADR

UN 1588 6.1/PG 2

RTECS

U8925000

Related Products

Replaced by

22,871-0 Sodium nitroferrocyanide(III) dihydrate ACS reagent, 99 %

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